CHAPTER 12

Reproductive Behavior and Sexual Selection in Annual Fishes

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12.1 INTRODUCTION

While the most noteworthy feature of annual fishes may be their unusual life history, these animals have long been appreciated by scientists and hobbyists for the striking sexual dimorphism and dichromatism they share with other cyprinodontiforms. Indeed, one of the first studies on sexual selection in fishes was on the African annual *Nothobranchius guentheri* (Haas, 1976a). Males of most annual fish species are larger than females, exhibit bright coloration in the body flanks and have strongly pigmented unpaired fins, perform elaborate courtship displays, and engage in ritualized aggressive contests with other males for access to mates. By contrast, females are small and cryptic with few distinguishing characteristics across species and express behavioral preferences for distinct male phenotypes (Figure 12.1). This pronounced sexual dimorphism suggests that sexual selection shapes the reproductive behavior in this clade (Box 12.1) and that direct and indirect competition for mates should be intense.

Furthermore, these fishes live in small, geographically isolated, and ephemeral pools. Thus, annual fishes provide a unique opportunity for empirical studies of the interaction between sexual selection and extrinsic variables like sex ratio and water quality, as well as of the role of sexual selection in the evolution of reproductive barriers among populations. Nonoverlapping generations and numerous disjunct populations make annuals an ideal system for integrating field data with theoretical predictions. Although courtship and mating behaviors have been described in detail in several species, it is only recently that we have begun studying sexual selection in this group of fishes systematically. Two very distinctive advantages are that we can rule out cross-generation learning (Verzijden et al., 2012) and that the short window for reproduction makes it ideal for understanding life history trade-off involving mating decisions. Moreover, from a

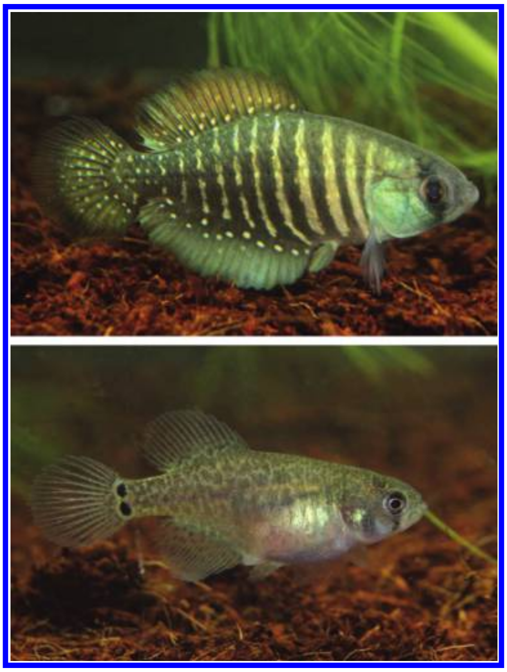

Figure 12.1 Male (top) and female (down) *Austrolebias charrua* (unscaled). (Photos by M. Casacuberta.)

BOX 12.1 SEXUAL SELECTION

Sexual selection is a powerful mechanism of evolutionary change proposed by Darwin in 1871 that results in differential reproductive success among individuals of the same sex and explains both the emergence and the evolution of sexually dimorphic traits. The extravagant male ornaments in many species are good indicators of the action of sexual selection. These ornaments are usually disadvantageous to a male's viability or survival, but they are advantageous in that they increase a male's mating success. Sexual selection is revealed through direct competition among members of the same sex (intrasexual selection) or by the choice of one sex over individuals of the other sex (intersexual selection); the relative importance of these two processes differs among species. In general, females invest a large amount of energy in egg formation, and their reproductive potential is often limited by resource availability. In contrast, the number of sperm that males can produce rarely limits their reproductive success. Consequently, females are most often selective in their mating decisions, whereas males usually actively compete for access to females or resources to attract them, and there is more variance in male than in female reproductive success (Andersson, 1994). Although the evolution of certain male traits can be promoted exclusively by one of the two components of sexual selection, they often act simultaneously (in concert or in opposition) because many traits are involved in both female mating decisions and male contest outcomes (Berglund et al., 1996; Candolin, 2004; Moore and Moore, 1999; Qvarnström and Forsgren, 1998).

In intersexual selection, individuals can base their choice on signals (often displayed during courtship behavior) that may or may not be associated with latent traits in potential mates (Andersson, 1994; Darwin, 1871). In some cases, the benefits of mate choice could be directly related to survival or fecundity. For instance, selection will favor the mating preferences for individuals with higher fertility, or who provide better quality or quantity of resources and parental care. More controversially, mate choice may be adaptive according to indirect benefits achieved by mating with certain individuals and thereby obtaining offspring with genotypes that increase the viability and survival ("good genes" model, Zahavi, 1975; but see Kirkpatrick, 1987). A more general mechanism for the elaboration of male traits and female preferences is Fisher's runaway model (Fisher, 1930), in which traits and preferences are favored merely as a consequence of the established genetic correlation between female preference and male trait (Andersson, 1994). Finally, mating preferences may have arisen independent of male traits, in response to a preexisting sensory bias that has evolved in a nonreproductive context (Endler and Basolo, 1998; Macías Garcia and Ramirez, 2005; Ryan, 1998). All of these mechanisms may act alone or, more likely, in concert to determine the evolution of mate choice and sexual characters (Kokko et al., 2003).

In a microevolutionary context, sexual selection affects population genetic structure, but also acts as a driving force of morphological and behavioral innovations linked to systems of mating signals. It can therefore have a leading role in the establishment and strengthening of barriers to gene exchange between populations, and therefore impacts speciation (Butlin and Ritchie, 1994) and hybridization (Rosenthal, 2012). Both theoretical and empirical studies (reviewed in Panhuis et al., 2001; Ritchie, 2007) have shown that sexual selection can drive speciation (due to reproductive isolation via the generation of assortative mating) by producing divergence between populations in sexual traits (female preferences and male signals). Sexual selection is considered one of the most crucial processes responsible for the generation and maintenance of biological diversity (Boake, 2002).

practical point of view, reproductive behavior can be observed easily, as both males and females perform reproductive activity daily.

Most research on sexual selection in annual fishes has focused on two systems: the *Austrolebias charrua—A. reicherti* system in Uruguay and *N. furzeri–N. orthonotus* system in Mozambique. Additional insight comes from work on captive populations of *N. korthausae*. Throughout the chapter, we will try to demonstrate the close relationship between reproductive behavior and particular life history of annual fishes, using examples from *Austrolebias* and *Nothobranchius*, the two most diverse genera of annual fishes. We will discuss the sexual signals involved in intrasexual competition, courtship, and mate choice. Finally, we will discuss the potential role of sexual selection on the divergence of this group of fishes.

12.2 INTRASEXUAL COMPETITION

In *Cynolebias* and *Simpsonichthys*, females aggressively suppress reproduction in other females; in an aquarium, one dominant female will exclude the others through chases and bites, and consequently only the dominant female spawns (Belote and Costa, 2002, 2003). In *Simpsonichthys,* females can even fight to the death (Belote and Costa, 2002). In contrast, there is no fight among females in *Austrolebias* and *Nothobranchius*, and two *Austrolebias* females may try oviposit with a single male at the same time (Belote and Costa, 2004; Haas, 1976b). In *Nothobranchius*, additional females may remain in close proximity to the spawning pair and spawn later with the male after ongoing oviposition is resumed, with no aggression among females observed (Haas, 1976b). This difference in female aggressive behavior among genera could be related to the fact that females of *Cynolebias* play an active role during the courtship behavior, while in *Austrolebias* and

Nothobranchius, females are limited to observing male courtship displays and to following males to spawn (see the following section).

Regarding male–male competition, *Austrolebias* males are very aggressive and quickly escalate to physical fights in aquaria. Experiments addressing social interactions in aquaria with restricted spawning areas have found that males of *A. reicherti* defend and exclude rivals from a spawning territory. In addition, both territorial residence and dominance are stable over time, although females move freely around the aquarium (Fabra, 2011). Social interactions likely establish dominance ranks that determine access to females and/or best areas to spawn in the heterogeneous conditions of temporary ponds they inhabit (Passos et al., 2013a). These findings also suggest that female choice plays an important role, since male coercion of females is limited. Future studies should address whether male–male aggression acts to physiologically suppress reproduction in losers as it does in other fishes (e.g., Desjardins and Fernald, 2008; Fernald, 2009).

Male–male competitive interactions in *A. reicherti* and *A. charrua* often begin with displays similar to courtship toward females: males exhibit lateral displays alternated with sigmoid displays and place their flanks together in parallel or antiparallel position while quivering (Passos et al., 2013a; Table 12.1). Conflicts are often resolved through mutual displays, with one fish retreating. If not, one fish usually charges and bites the other male, often targeting the fins. The bites can be brief or may last several seconds when the aggressor uses his jaws to seize his opponent, causing evident injuries to the fins. Sometimes the fish receiving the first attack retreats and the contest is resolved; at other times the fish under attack fights back and the contest escalates. The outcome of the contest is clearly indicated by morphological and behavioral differences between the contenders. The subordinate male "turns off" quickly, decreasing the intensity of coloration, reducing the contrast of flanking vertical bars, and folding the dorsal fin. A defeated male resembles a female, confusing even an experienced observer. Once dominance is established, the subordinate male remains mostly

Table 12.1 Behavioral Unit Description of *Austrolebias*

Behavioral Units	Context	Description
		Males
Lateral display	Courtship and agonistic interactions	Body still, dorsal and anal fins extended, with or without quivering
Sigmoid display	Courtship and agonistic interactions	Lateral display with body undulations
Invitation to follow	Courtship	Successive short swimming away and stops
Invitation to dive	Courtship	Body in vertical or oblique position, with head over the substrate, and quivering of extended dorsal and anal fins
Mating	Courtship	Body placed by the side of female, pressing its body to the substrate. Unit performed from near the surface to completely buried
Lateral display with contact	Agonistic interactions	Lateral display, above, with both actors touching in a parallel or antiparallel position
Attack	Agonistic interactions	Actor bites or attempts to bite recipient, often directed at the fins
Submission	Agonistic interactions	Rapid flight to sides or corner, accompanied by color loss and folding of the dorsal fin
		Females
Following acceptance	Courtship	Female follows male (displaying IF)
Diving acceptance	Courtship	Females placed by the side of male, touching its body
Mating	Courtship	Female buried in the substrate (partially or completely)

Source: Adapted from García, D., M. Loureiro, and B. Tassino. 2008. *Neotropical Ichthyology* 6: 243–248; Passos, C. et al. 2013a. *Behavioral Process* 96:20–26.

inactive and flees the dominant male when approached. In contrast, a dominant male has darker coloration, enhancing both body and fin pigmentation design, unfolds his dorsal fin, and moves freely around the aquarium, performing aggressive displays against the other males. Once the hierarchy is established, the overall level of aggression decreases, and the dominant male's lateral and sigmoid displays are the most frequently observed agonistic interactions (Passos et al., 2013a).

In the laboratory, the hierarchy between individuals is stable for several days, even though coloration, fin deployment, and behavior are dynamic and can change in few seconds. The "turn on" coloration likely signals social status and acts as a reliable indicator of the competitive abilities of a male, in the sense that "dishonest" individuals will be exposed to fight escalation (Maynard Smith and Harper, 1988). Sexually dimorphic pigment patterns in other fish species exhibit rapid changes with aggression and social status (Hurd, 1997; Morris et al., 1995; Nelissen, 1991). Chemical cues may also play a role in short- and long-term dominance interactions (Almeida et al., 2005; Barata et al., 2007).

In *Nothobranchius*, males also engage in fierce fights, with the level of male–male aggression varying across species (Polačik and Reichard, 2011; Reichard et al., 2014). It is not clear whether males patrol territories in the wild, but the dominant male usually guards the spawning site with a substrate in an aquarium and repels other males from its vicinity (Reichard and Polačik, 2010). The bottom of *Nothobranchius* pools is typically more uniform than those of *Austrolebias* (see Chapter 9); substrate suitable for spawning and egg development is distributed across the entire pool. At least under captive conditions, dominant males have the highest mating success due to exclusive access to prime spawning sites; in a study under seminatural conditions, spawning only took place in deeper parts of the pool (Haas, 1976b). Males actively explore their home range and search for females, with dominant males apparently controlling the largest areas. In the wild, dominant males likely become most conspicuous to females by moving freely around a pool and enjoy the most frequent female encounters and mating. Dominant males also actively disrupt the spawning of other males (Haas, 1976b).

Male–male combats are comparable to *Austrolebias*. Males rapidly approach each other and perform lateral displays using the spread of unpaired fins. Unlike *Austrolebias* males, male *Nothobranchius* prominently extend the lateral projections of their opercular and branchiostegal membranes (Haas, 1976b). The branchiostegal membrane is exceptionally well developed in males and conceivably has an important function in agonistic signaling similar to other fishes, for example, the Siamese fighting fish, *Betta splendens* (Rosenthal and Lobel, 2006). The lateral displays include tail beating and attempts to bite an opponent's fins and flanks, in addition to mutual threats performed with the branchiostegal membranes prominently displayed (Haas, 1976b). When a contest is not resolved with these displays, males may lock their jaws and remain in the locked position for several seconds to minutes. At least in aquaria, males rapidly establish linear hierarchies (Haas, 1976b), and male–male aggression subsides to a relatively low level. There are major interspecific differences in the intensity and frequency of male–male aggressive interactions, with large species such as *N. orthonotus* being more aggressive than smaller species, for example, *N. pienaari* and *N. korthausae* (Polačik and Reichard, 2009, 2011). A slight increase in male–male aggression associated with the peak of mating activity is observed during the midday hours, even in the established hierarchies (Haas, 1976b).

Dominant males have brighter coloration than subordinates. However, in contrast to *Austrolebias*, changes in male coloration are not abrupt and do not respond rapidly to the subtleties of social situations. Perhaps this is because the changes in coloration do not involve melanocyte-related and carotenoid patterns, which are known to be involved in quick responses to individual status (Kodric-Brown, 1998). Instead, *Nothobranchius* male coloration is dominated by structural colors (Ng'oma et al., 2014), and its intensity is likely mediated hormonally rather than neuronally.

As in other fishes (Beaugrand et al., 1996; Benson and Bassolo, 2006; Moretz, 2003), larger *Austrolebias* males are more aggressive and socially dominant (Passos et al., 2013a), though the association between the body size and dominance is much weaker in *Nothobranchius* (Polačik and Reichard, 2009; Reichard and Polačik, 2010). Fight duration between males of *A. charrua* decreases with increasing differences in the body size of opponents (Passos et al., 2013). When the size difference is large, dominance is established by lateral and sigmoid displays, without escalation. Lateral displays, in which one individual exposes his flank to the opponent with fin extension and oscillation, and likely provide the receiver with visual and mechanosensory information about the body size of the sender (Bleckmann, 1993). Sigmoid displays, in which an individual performs high-speed undulating body movements, may be predictors of the body size, motivation, and locomotor performance (Passos et al., 2013a).

Several researchers have suggested that a negative relationship between contest duration and body-size asymmetry indicates mutual assessment in animal contests (e.g., Bridge et al., 2000; Enquist et al., 1990; Hack, 1997). In mutual assessment models, individuals assess their own body size relative to their opponent, and contest duration is expected to be inversely correlated with the relative body size of contestants, because an animal can detect and act on a large difference quicker than on a small difference (Enquist and Leimar, 1983; Maynard Smith and Parker, 1976). However, recent studies have shown that this result is also consistent with a self-assessment model (Prenter et al., 2008; Taylor and Elwood, 2003), in which animals make decisions based on their own fighting skills. Fighting is costly in terms of time and energy, risk of physical injury, and predation. Furthermore, selection should favor any mechanism allowing accurate gathering of information on the likely costs and benefits of continuing a conflict and to enable appropriate tactical decisions (Maynard Smith and Parker, 1976; Parker, 1974).

In several taxa, including many fishes, males are polymorphic. Intraspecific polymorphism typically involves differences in coloration among the morphs (i.e., polychromatism; see Gray and McKinnin, 2007). Male coloration may be continuous, but overall distinct morphs are often clearly separated. In several *Nothobranchius* species, males have two discrete color morphs, while other species show a continuous gradation of male coloration across populations (Wildekamp, 2004). The genetic basis of discrete polymorphism for some species is understood and relatively simple. In *N. furzeri*, the allele for yellow color is dominant, whereas the red allele is recessive (Valenzano et al., 2009). The role of such dimorphism is important in the establishment of male hierarchies; in many systems, color phenotypes predict dominance in male–male contests (Pryke and Griffith, 2006), and dominant males are preferred by females or mate by coercion. In contrast, other color morphs are subordinate in hierarchies and mate largely by alternative tactics (Sinervo and Lively, 1996). Recent experiments with *N. furzeri* revealed that male coloration (red or yellow caudal fins) has no impact on the outcome of dominance hierarchies, at least in simple dyadic combats (Reichard et al., unpublished data).

12.3 COURTSHIP SEQUENCE AND SEXUAL SIGNALS

Courtship and mating behavior have been described in a number of species, notably in *Austrolebias* (*A. bellottii*: Vaz-Ferreira et al., 1964; Vaz-Ferreira and Sierra, 1972; *A. nigripinnis*: Belote and Costa, 2004; Vaz-Ferreira et al., 1964; Vaz-Ferreira and Sierra, 1972; *A. viarius*: Vaz-Ferreira et al., 1964; Vaz-Ferreira and Sierra, 1972; *A. luteoflammulatus*: Vaz-Ferreira et al., 1964; Vaz-Ferreira and Sierra, 1972; *A. cheradophilus*: Vaz-Ferreira et al., 1964; *A. cyaneus*: Belote and Costa, 2004; *A. charrua*: Belote and Costa, 2004; *A. reicherti*: García et al., 2008; and *A. affinis*: Libran, 2011) and *Nothobranchius* (*N. guentheri*: Haas, 1976a,b; *N. korthausae*: Polačik and Reichard, 2009; Reichard and Polačik, 2010; *N. furzeri*: Polačik and Reichard, 2011; and *N. orthonotus*: Polačik and Reichard, 2011). Reproductive behavior is highly conserved in the genus *Austrolebias*; all species exhibit similar courtship displays with addition or slight modification of the same behavioral units (Table 12.1). Other South American annuals also use the same basic behavioral patterns (e.g., *Simpsonichthys* and *Cynolebias*; Belote and Costa, 2002, 2003). In contrast, courtship displays in *Nothobranchius* are relatively simple (Haas, 1976a; Reichard and Polačik, 2010).

Courtship sequences for *A. reicherti* and *A. charrua* have been described in detail (Belote and Costa, 2004; García et al., 2008; Passos, 2013; Table 12.1; Figure 12.2). A courting male typically follows a female and begins to display when he comes within her field of view. Male courtship consists of lateral displays with rapid quivering and expanded fins, alternating with sigmoid displays with fast body undulations. Subsequently, the male darts a short distance away and makes a new sequence of lateral and sigmoid displays. If the female is responsive, she approaches and follows the male, who swims near the bottom searching for a suitable site for oviposition. Then, the male places his snout into the substrate in a 45° to 90° angle and then vigorously undulates his body. If the female does not follow the male, then the male quickly rises and restarts lateral and sigmoid displays. Alternatively, the female comes to the side of the male and touches his flank in a parallel position, in which case the pair digs under the substrate while quivering. Oviposition and fertilization occur in the substrate, with the male pressing the female against the substrate with his body lying over female (Table 12.1; Figure 12.2). After a few seconds, the couple emerges, either together or at different times, and a new courtship cycle starts. The duration of the courtship and the frequency of spawning depend mainly on the whether the female stays with the male and whether there is a presence of competitors. It is not unusual for a male to abandon courtship and switch to confront a rival male. However, if the couple is kept isolated from other individuals, the courtship continues until the female becomes unresponsive. Usually, females of most annual fishes do not show elaborate behaviors during mating; they mostly observe the courtship display of the males and follow a male to a spawning site. However, the female of *C. albipunctatus* touches its snout to the urogenital region of the male, sometimes exhibiting brief and subtle courtship-like waving movements (Belote and Costa, 2003).

In *Nothobranchius*, the spawning sequence is very similar across species. Males initiate mating, but females sometimes approach a male first, especially under experimental conditions of physical isolation behind a barrier (Reichard and Polačik, 2010). The male approaches a female and

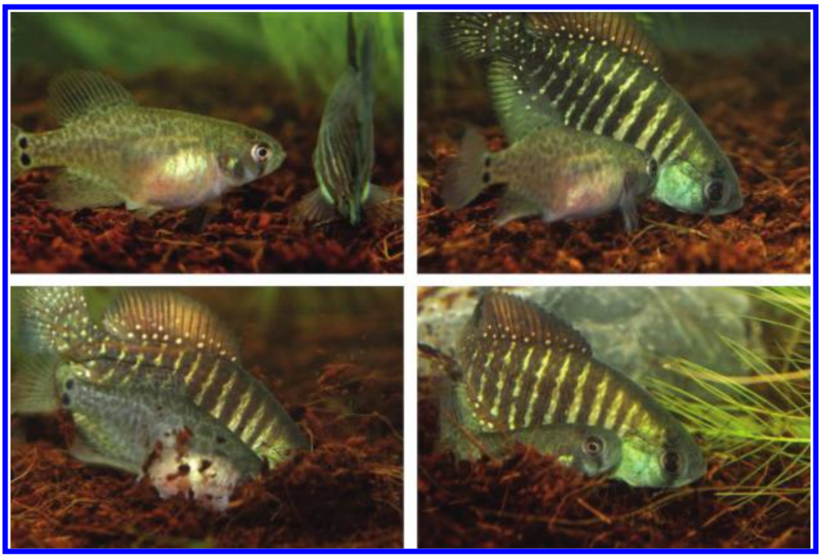

Figure 12.2 Courtship sequence of *Austrolebias charrua*: male display (top left), invitation to dive (top right), diving acceptance (down left), and mating (down right) (see Table 12.1). (Photos by M. Casacuberta.)

Figure 12.3 Spawning behavior of *Nothobranchius eggersi*: male folds female using his dorsal and anal fins, and female stiffens her anal fin (left); the male then raises his head and pushes female toward substrate (right); this is followed by oviposition. (Photos by K. Zahrádka.)

frequently stops to engage in displays. At this stage, a female unwilling to spawn swims away, while a receptive female allows the male to approach. The male makes displays laterally to the female but without spreading his fins, as occurs during agonistic encounters (Haas, 1976b). Individual lateral displays are interrupted by sequential approaches, with the two behaviors being swapped in succession. In *N. korthausae*, the male's head points slightly upward at an angle of approximately 30° to 45° during displays (Reichard and Polačik, 2010). Alternation of displays and approaches results in a darting approach movement of the male toward the female, which is followed by physical contact. The male places his lower jaw on the upper part of the female's head or slightly behind, directing her toward a spawning site. Then, the male moves slowly alongside the female, keeping physical contact with her and clasping her from above using his dorsal fin. Maintaining physical contact, the male raises his head and pushes his caudal fin away from the female with rapid flicking of the pectoral fins. He folds the female using his anal fin (Figure 12.3), and the female stiffens the anterior part of her anal fin (possessing rigid prolonged rays) and inserts it into the substrate. The pair remains motionless for few seconds, followed by oviposition of a single egg on the substrate during a jerked downward movement. This final phase disturbs the substrate, and oviposition can thus be easily recognized. The pair may return to clasping, and the second part of spawning sequence is finalized by another oviposition (Haas, 1976b; Reichard and Polačik, 2010). Haas (1976b) observed two to seven successive ovipositions in *N. guentheri*, but the series can be longer, at least under experimental conditions, in *N. furzeri* (Blažek et al., 2013).

The sexual dimorphism of male form and color, coupled with intensified contrast of body markings during courtship, suggests that visual cues play an important role during reproductive interactions in annual fishes. There is, however, limited experimental evidence for the role of visual signals in mate choice (see the following section).

Males of *A. reicherti* produce chemical cues during courtship that cause behavioral changes in conspecific females (Passos et al., 2013b). Chemical cues were obtained by placing individual males next to individual females for 24 h to elicit courtship. Females preferred male-conditioned water when tested both against control water and female-conditioned water (Figure 12.4a). In fishes, semiochemicals have been shown to play an important role in mate choice (Fisher and Rosenthal, 2006; Milinski et al., 2005), intrasexual competition (Almeida et al., 2005; Barata et al., 2007), and speciation processes (Plenderleith et al., 2005). Olfactory cues may also play particularly important roles in recognition of conspecifics and hence act as a mechanism of reproductive isolation among closely related species (Kodric-Brown and Strecker, 2001; McLennan and Ryan, 1997, 1999; Rafferty and Boughman, 2006; Wong et al., 2005). This has been convincingly shown in swordtails (Poeciliidae), which have been extensively studied for mate choice and species recognition mechanisms (Crapon de Caprona and Ryan, 1990; Fisher et al., 2006, 2009; McLennan and Ryan, 1997, 1999; Wong et al., 2005). Sympatric *Xiphophorus* species use chemical signals for species recognition, and the

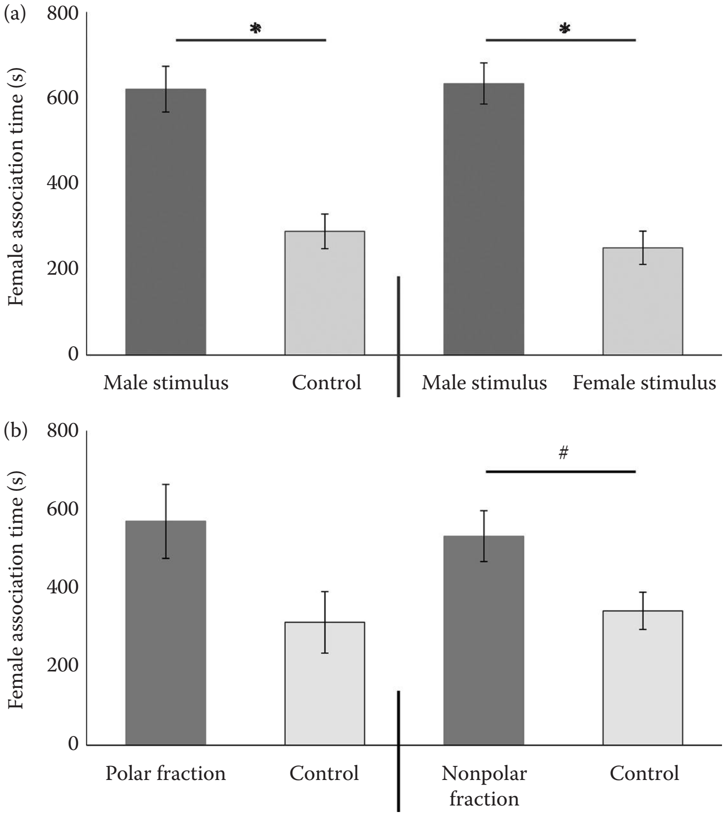

Figure 12.4 Association time (mean ± SE) of females *Austrolebias reicherti* with chemical cues. Male-conditioned water vs. control water, and male- vs. female-conditioned water (a); polar fraction of the male-conditioned water vs. control water, and nonpolar fraction of the male-conditioned water vs. control water (b) (*$p < 0.05$; #$0.05 < p < 0.1$). (Modified from Passos, C. et al. 2013b. *Ethology* 119: 891–897.)

disruption of chemical communication results in interbreeding of natural populations of swordtails (Fisher et al., 2006; Rosenthal et al., 2003). Chemical cues in *Austrolebias* could be important during the initial stages of mate location and recognition, when distance, occluding vegetation, and turbidity within the pond would limit visual detection. This is also pertinent to *Nothobranchius*, which often inhabit pools with very turbid water, but the role of chemical cues in *Nothobranchius* has not been investigated. Also, chemical cues could reinforce visual cues at close proximity, for example, during courtship.

Characterization of the chemical signals in annual fishes will likely be a challenging task. Specialized scent glands or overt marking behaviors are not common in fishes. Consequently, it is difficult to obtain clean samples for chemical analysis. Indeed, in some species, it has been shown that courting males release their pheromones in the urine, which increases the chemical complexity of the samples (Maruska and Fernald, 2012; Rosenthal et al., 2011). Most fish pheromones that have been chemically identified to date comprise prostaglandins and steroids, but there have been reports of a communicative role in bile acids and amino acids (reviewed in Sorensen and Hoye, 2010). These compounds differ in their polarity; therefore, a crude separation of polar and nonpolar compounds, combined with activity bioassays, would provide primary information as to the chemical nature of an olfactory cue. In *A. reicherti*, female preference for male cues was reduced or abolished when cues were separated into polar and nonpolar fractions. However, a tendency was observed for attraction toward the organic fraction that included compounds of medium polarity (Passos et al., 2013b; Figure 12.4b). Alternatively, polar and nonpolar compounds may combine to produce a multicomponent cue that is attractive to females (Passos et al., 2013b). Indeed, in goldfish, *Carassius auratus,* both polar and nonpolar fractions of pheromone blends were necessary to achieve biological activity (Levesque et al., 2011).

In addition, other modalities may play a role. The undulating movements and quivering of a courting male generate currents, and receivers could simultaneously attend to chemical, visual, and mechanosensory information. Vibratory and undulatory movements of males during courtship are common in many fishes (Rosenthal and Lobel, 2006). Several studies have reported that these displays produce low-frequency mechanosensory cues (Bleckmann, 1993; Nelissen, 1991; Sargent et al., 1998) detectable by the lateral line system of the receiver (Bleckmann, 1993). Although mechanosensory communication in fishes has received little attention, there is compelling evidence to suggest that this modality could play an important role in courtship behavior and mate choice (e.g., Satou et al., 1994a,b). Annual fishes have a highly developed laterosensory system (Belote and Costa, 2003; García et al., 2008), and the close-range vibrations and motor displays performed during courtship would generate hydrodynamic cues that could be detected by the females.

Finally, many other fishes use sound in the context of courtship and mating. While sound production is taxonomically widespread (reviewed in Bass and McKibben, 2003), the best evidence for the role of acoustic cues in mate choice comes from cichlids (Maruska et al., 2012; Verzijden et al., 2010). Differences in courtship sounds among sympatric cichlid species may contribute to reproductive isolation and speciation (Amorim et al., 2008; Danley et al., 2012; Lobel, 1998). At least two South American annual fishes produce sound: *Cynolebias albipunctatus* (Belote and Costa, 2003) and *C. parnaibensis* (Costa et al., 2010). The mechanism of sound production in these species is not known but may involve the pharyngeal jaw apparatus (Belote and Costa, 2003; Costa et al., 2010). The bony support of the gill arches in these fishes is highly modified (Costa, 2001) and skeletal traits may be related to sound production (e.g., friction of teeth, Belote and Costa, 2003).

Sound production is also plausible in *Nothobranchius*. During experimental work with *N. korthausae*, a female apparently responded to the initial stage of male courtship behavior (i.e., darting approach) with an approach, despite the male being visually and chemically isolated in an adjacent aquarium (Reichard and Polačik, 2010). Intriguingly, the sulcus (a specialized part of the saccular otolith in the inner ear of fishes) morphology is divergent in sympatric *Nothobranchius* species (Reichenbacher and Reichard, 2014). The sulcus is in contact with a sensory epithelium (sensory hair cells), and its species-specific morphology may indicate specialization in hearing abilities (Fekete, 2003). Species-specific characteristics of the sulcus were confirmed in both tested pairs of sympatric species, *N. rubripinnis* and *N. ruudwildekampi* from coastal Tanzania and *N. orthonotus* and *N. furzeri* from Mozambique (Reichenbacher and Reichard, 2014). Intraspecific auditory communication would have obvious advantages for annual fishes that often live in very turbid waters. Additional work may reveal a role for acoustic communication in courtship and mating.

12.4 MATE CHOICE

The sexual dimorphism and small size of annual fishes make them a good model for laboratory studies of sexual selection (Box 12.1). Surprisingly, apart from one early study on *N. guentheri* (Haas, 1976a), mate choice has not been addressed until recently. When given a choice between two males varying in size, females of both *A. charrua* and *A. reicherti* prefer to mate with larger males (Passos et al., 2013a, 2014). The preference for larger males is shared with many fishes (Andersson, 1994), including other cyprinodontiform fishes (e.g., MacLaren and Daniska, 2008; Ptacek and Travis, 1997; Rosenthal and Evans, 1998; Ryan et al., 1990). Several nonexclusive factors may favor the evolution or maintenance of preferences for large size (Box 12.1). First, larger males could generate great visual stimulation and therefore elicit sensory bias underlying female preferences (Rosenthal and Evans, 1998). Second, female preference could also evolve via indirect selection on male traits (Andersson, 1994; Fisher, 1930; Zahavi, 1975). Third, females may obtain direct benefits by mating with larger males, since body size is correlated with dominance status (see above). In South American annual fishes, direct benefits of mating with dominant males could include additional spawning sites and refuges, which may be limiting factors for reproduction and viability of eggs. In contrast, female choice in *Nothobranchius* is independent of male body size, except when the difference between males is relatively large (Reichard and Polačik, 2010), though the effect of male body size has not been tested directly.

Male–male interactions may be a source of information in mate choice (Candolin, 1999; Doutrelant and McGregor, 2000). Direct experimental evidence in fishes indicates that witnessing male–male interaction influences subsequent female choice (Cox and LeBoeuf, 1977; Montgomerie and Thornhill, 1989), although sometimes females prefer losing males (Wong and Candolin, 2005). Indeed, socially dominant males may often be worse at providing direct benefits like nest defense (e.g., Wong, 2004) and may behave aggressively toward females (Reichard et al., 2007). The interaction between male–male competition and female choice in *A. charrua* was tested experimentally (Passos et al., 2013a). Females were given a simultaneous choice between two males and consequently witnessed agonistic encounters between the males. Subsequent testing showed that females were consistent in their choice and selected the same male in both tests, independent of whether they had observed the male contest or not, suggesting that competition does not increase the attractiveness of the dominant male over the subordinate (Passos et al., 2013a). However, given the difficulty of disentangling the highly correlated effects of body size and social hierarchy (see above), it is not possible to conclude that female perception of male dominance in *A. charrua* is irrelevant in female choice. A possible approach to distinguish the effects of body size from the effects of social hierarchy would be to engineer dominance relationships among males, such that the smaller male is dominant. This could be achieved through the effect of prior social experience on agonistic behavior, in which individuals modify their behavior based on the outcome of previous contests. There is evidence that individuals who were winners in a fight increase their chances to win in the future (winner effect), while losing individuals decrease their chances to win in other contests (loser effect) (Hsu et al., 2006). Therefore, previous agonistic trials could reverse the outcome of the interaction between competitors, such that the smaller male wins. Moreover, field studies should address the relationship between male aggression and egg viability.

Females of *N. korthausae* did not lay more eggs with socially dominant males. In noncompetitive trials, in which males with previously ranked dominance were sequentially presented to females with no contact to any rivals during the observation, females did not discriminate among males on the basis of their dominance or body size. There were also no benefits to mating with large or dominant males in terms of increased fertilization or hatching success in aquaria (Reichard and Polačik, 2010). In choice trials with *N. korthausae*, females made their partner choices, but these were not related to male dominance or body size. In these trials, however, male dominance was tested after completion of female choice trials. Therefore, females were unable to make decisions on the basis of “eavesdropping” on male contests, and males were unable to modulate their display behavior according to winner/loser effect (Polačik and Reichard, 2009).

Several studies have documented mate choice based on visual displays of male coloration in cyprinodontiform fishes. In swordtails, females attend to vertical bars on the lateral surface of males, which intensify during courtship and aggressive interactions (e.g., Morris et al., 1995; Morris et al., 2003). *Austrolebias* males have similar vertical bars, but there is no detectable relationship between the number, width, or intensity of bars and female mating preference (Passos, 2013). Techniques like computer-animation playback (Culumber and Rosenthal, 2013; Rosenthal, 2000) could be useful in isolating preferences for signal components.

In *N. guentheri*, females prefer to associate with brighter males (Haas, 1976a). However, perhaps surprisingly, discrete male morph (red or yellow caudal fin) had no effect on female choice in *N. furzeri*. Given the potential for assortative mating and inability to phenotypically score female color alleles, females were tested twice, each time with a different pair comprising a red and a yellow male. Females were choosy and associated with one male preferentially, but their preference was not consistently related to male color morph. A female often preferred a male of one color in her first trial, but a male of the other color morph in the second trial. Red and yellow males in this experiment were wild captured and originated from a single population (Reichard et al., unpublished data). In *N. korthausae*, males of red and yellow morphs came from allopatric populations, and experimental fish were descendants of already captive fish. Virgin females mated indiscriminately, but nonvirgin females from both populations preferred red males (that were sympatric for females from one population but allopatric for females from a second population). Red males displayed to females at a disproportionally higher rate; it cannot be separated whether females responded to red coloration, cues from other sensory modalities, or higher courtship intensity (Reichard and Polačik, 2010). The role of discrete male color morphs on mate choice in *Nothobranchius* requires further investigation. Ideally, future studies should include sympatric and allopatric populations and control samples for a potential effect of inbreeding in captive populations, since female choice for optimal outbreeding (e.g., detected by chemosignals) may override preferences related to coloration (Agbali et al., 2010).

Female mate choice is a contextual phenomenon modulated by different factors (Jennions and Petrie, 1997). The fitness effects for females of choosing or of mating with certain males may vary in magnitude and direction as environmental conditions or individual phenotypes change (Borg et al., 2006; Forsgren et al., 2004; Jennions and Petrie, 1997; Qvarnström, 2001). Mate choice can be influenced by several factors, for example, availability of resources (Fisher and Rosenthal, 2006; Moskalik and Uetz, 2011), intensity of intrasexual competition (Lehtonen and Lindström, 2008), and individual condition (Amundsen and Forsgren, 2003; Burley and Foster, 2006; Hunt et al., 2005). One important variable is the operational sex ratio (OSR; Owens and Thompson, 1994), defined as the ratio of males and females available to mate at a given time. Given daily reproduction of male and female annual fishes, the OSR generally equals the adult population sex ratio. Natural populations of *A. charrua* and *A. reicherti* vary markedly in OSR over the course of one season. At the beginning of the reproductive season, the OSR did not deviate from parity, whereas later in the reproductive season the OSR became female biased (Passos, 2013; Passos et al., 2014). In captivity, we have not detected any sex differences in mortality rate or biases in the adult sex ratio, suggesting that increased extrinsic mortality in males could lead to the observed biases in the OSR at the end of the season in natural populations. *Austrolebias* species exhibit intense male–male competition, often involving serious injuries and elaborate male courtship displays during reproduction (see above). A plausible explanation is that high male mortality stems from physical exhaustion resulting from courtship displays and male–male competition (Passos et al., 2014). Furthermore, males exhibit brighter coloration and show sexual displays that may incur an increased predation risk. Female-biased sex ratio in wild populations is also widespread in *Nothobranchius*, in which predation from predatory birds and hemipterans were implicated as potential sources of male-biased mortality (Haas, 1976a; Reichard et al., 2014). Mortality directly related to costly male–male interactions is also plausible in *Nothobranchius* (Reichard et al., 2014). Intriguingly, in both African and South American annual fishes, adult sex ratio often stabilizes at a ratio of 1 male to 2 females (Passos et al., 2014; Reichard et al., 2014), and the same adult sex ratio is reported for other small fishes with striking sexual dimorphism (Arendt et al., 2014).

In concert with the change in the OSR, female choosiness changes during the reproductive season in *A. reicherti* (Passos et al., 2014). At the start of the reproductive season, females strongly preferred larger males in simultaneous choice trials. In addition, the strength of female preference, measured as the difference in association time between stimuli, was positively related to the

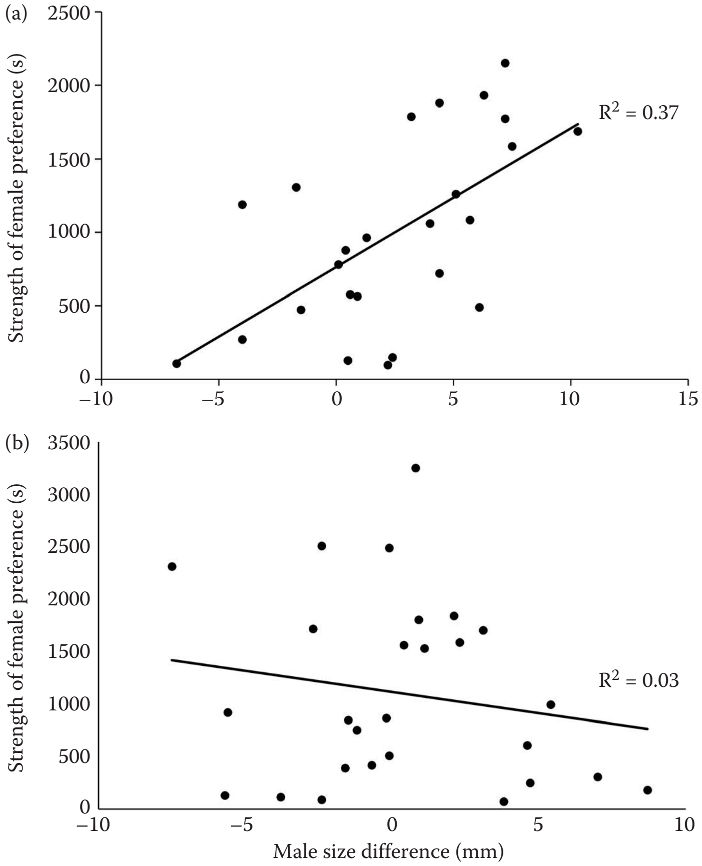

Figure 12.5 Female mate choice and male body size. Relationship between male size difference (standard length of preferred male—standard length of nonpreferred male) and strength of female preference (difference in time spent with preferred and nonpreferred males) in the annual killifish *Austrolebias reicherti* at the beginning (a) and end (b) of the reproductive season. Negative scores indicate more time with smaller males, positive scores indicate more time with larger males. (Modified from Passos, C. et al. 2014. *PLoS ONE* 9: e101648.)

asymmetry in male body size. However, female preference for larger males vanished entirely at the end of the reproductive season. Females were equally likely to choose small or large males later in the season, and female preference was not affected by the size difference between stimulus males (Figure 12.5). Different alternatives, not mutually exclusive, could explain this change (Passos et al., 2014). First, the reduction in choosiness could arise from diminished reproductive prospects due to a decline in male availability (Borg et al., 2006; Kokko and Johnstone, 2002). As the sex ratio becomes female biased late in the season, the opportunity for female choice may be reduced by a decline in rate of encounters with males (Kokko and Mappes, 2005). Second, the distinctive life history of annual fishes, where both life expectancy and mating opportunities decline rapidly over time, could account for the seasonal change in female choice. At the end of the reproductive season, all adults die due to extreme abiotic conditions. As this critical time approaches, a female may be faced with a trade-off in which she will have to decrease choosiness or risk losing the opportunity to fertilize her eggs. Finally, while female preference for body size is reduced over the course of the season, it is not necessarily the case that females become entirely nonselective. Females may assign varying weights to different male traits over the course of the season (Borg et al., 2006;

Forsgren et al., 2004). In *N. korthausae*, virgin females mated indiscriminately, while nonvirgin females of the same age were more selective. While this change in female choosiness runs counter the pattern in *Austrolebias*, indiscriminate mating of virgin females may have been due to a decrease in choosiness arising from their elevated motivation to spawn (Moore and Moore, 2001).

The fitness consequences for females of mating with certain males may vary throughout the season; females might then be expected to change how they respond to multiple male cues as environmental conditions or individual phenotypes change (Candolin, 2003; Jennions and Petrie, 1997; Qvarnström et al., 2000). Larger males have a competitive advantage in male–male competition (Passos et al., 2013a), and females could obtain direct benefits from mating with them early in the reproductive season to gain access to preferred areas for oviposition. However, the advantages of male size and the benefits to females of choosing large males may be reduced late in the reproductive season, because lower male density may decrease the intensity of male–male competition. The reduced direct benefits are a potential driver of the lack of preference for male size at the end of the reproductive season. Therefore, female choice could be based on other cues if traits other than male size become relatively more important for female reproductive success later in the reproductive season (Passos et al., 2014). Moreover, the extremely variable habitat provided by temporary ponds exposes fishes to drastic changes in water quality (particularly turbidity), and females may use different sensory modalities in mate choice across the life cycle. Future work will need to address whether females attend to different male traits over the course of a season and to disentangle the mechanisms leading to female behavioral changes, notably direct responses to demographic factors, environmental cues, or intrinsic changes during senescence. The general characteristics of *Austrolebias*, especially those related to their life cycle under great environmental and demographic changes, make annual fishes an exceptional and innovative model to contribute to the overall understanding of seasonal variation in mate choice.

There is also the potential for mate selection in males. Though traditionally it is argued that males are not selective and maximize reproductive success by increasing the number of copulations versus quality of partners (Bateman, 1948), theoretical approaches also suggest the occurrence of mutual choice even in species where each sex conforms to conventional roles (Bergstrom and Real, 2000; Edward and Chapman, 2011; Kokko and Johnstone, 2002). Male guppies (Herdman et al., 2004) and swordtails (Wong et al., 2005) show mating preferences despite females being the limiting sex. In fishes, bigger is often better from the point of view of males choosing females (e.g., Côté and Hunte, 1989; Pelabon et al., 2003). The reason is that female fecundity is typically an increasing function of body size (Charnov, 1993); this is indeed the case in *A. vandenbergi* (Schalk et al., 2014), *Cynopoecilus melanotaenia* (Gonçalves et al., 2011), and *N. furzeri* (Vrtílek and Reichard, 2015). There may therefore be a benefit to males of mating with larger females, although male preference remains to be evaluated in this system.

12.5 REPRODUCTIVE BEHAVIOR AND SPECIATION

The understanding of how new species arise, how they remain separate from other species, and how distinct species exchange genes are all major goals of current research in evolutionary biology. According to the biological species concept (Mayr, 1963), species are considered to constitute pools of interbreeding individuals that are substantially reproductively isolated from other such pools. Reproductive isolation is manifested through reproductive isolating barriers that decrease the probability of offspring production between individuals from different pools. The central problem of speciation is therefore the study of how reproductive isolation evolves to prevent actual or potential gene flow among populations. Its solution requires identification of the traits involved in the reduction of gene flow among populations and understanding of the evolutionary forces that have acted on these traits (Coyne and Orr, 2004). One of the most significant recent developments in speciation theory has been the increased attention given to sexual selection as an evolutionary force capable of rapidly inducing reproductive isolation among populations (Box 12.1). Although long recognized as important in intrapopulation evolution, sexual selection has more recently been invoked as a key promoter of both the origin and maintenance of new biological species (Kraaijeveld et al., 2011; Maan and Seehausen, 2011; Panhuis et al., 2001; Reding et al., 2013; Ritchie, 2007). Sexual selection can cause the rapid divergence of sexually dimorphic traits associated with mate acquisition (Andersson, 1994; Fisher, 1930). Many of these traits are involved in mate recognition and result in assortative mating within and among species (Coyne and Orr, 2004). Therefore, it has been proposed that sexual selection can facilitate divergence of mate-recognition systems among populations and thus, incidentally, lead to speciation (review in Panhuis et al., 2001; Ritchie, 2007).

Both theoretical (Higashi et al., 1999; Lande, 1981; Pomiankowski and Iwasa, 1998; Schluter and Price, 1993; Turelli et al., 2001; West-Eberhard, 1983) and empirical studies (e.g., Alexander and Breden, 2004; Boul et al., 2007; Seehausen and van Alphen, 1998; Seehausen et al., 1997) have shown the potential of sexual selection to contribute to the formation of reproductive barriers. Comparative studies have also suggested that sexual selection can contribute to and accelerate speciation (reviewed in Ritchie, 2007). Sexual selection is thought to have played an important role in major adaptive radiations, for example, Hawaiian *Drosophila* (Boake, 2005) and East African cichlid fishes (Seehausen et al., 1997).

Annual fishes constitute a useful model system for studying the role of sexual selection in reproductive isolation and speciation. The natural habitats of these fishes are temporary ponds that can be isolated from one another fairly easily, so interruption of gene flow among populations occurs quite often (Bartáková et al., 2013), though ponds may also be periodically connected during major floods. The potential repercussions of sexual selection for divergence and speciation are especially evident in geographically isolated populations, since sexual selection can drive the evolution of signaling and preference traits in divergent directions and thus result in prezygotic reproductive isolation among populations. On the other hand, those cases in which sexual selection has led to speciation should be characterized by significant differentiation in male sexual signals (Ritchie, 2007). In the "*A. adloffi*" species group, species differ almost exclusively in male coloration, while the general morphology and female coloration are quite uniform (Costa, 2006; Loureiro, 2004). Until now, there have been no indications of a notable ecological differentiation within of the "*A. adloffi*" species group, and molecular phylogenetic studies of this group showed that species divergence likely occurred recently through a burst process of multiple simultaneous speciation (García, 2006). This pattern is consistent with arbitrary divergence of male traits and female preferences via a Fisherian runaway process (Lande, 1981).

Within this group, *A. reicherti* and *A. charrua* in Uruguay have parapatric distributions along the southwestern lowlands of Laguna Merín, with a contact zone in the lower basin of the Río Cebollati (García et al., 2009), and hybrids are found wherever these species come into contact (Passos et al., unpublished data). Like other species in the group, *A. reicherti* and *A. charrua* are similar in morphology and behavior as well as in female coloration; however, they are strikingly different in male coloration, exhibiting divergent patterns of pigmentation of unpaired fins and in the design of dark vertical bands on body flanks (Loureiro and García, 2008). In *A. reicherti* males, the dark bands are narrower or equal in width to the lighter spaces between bands, and the unpaired fins are uniformly pigmented with a vertical black line on the posterior edge of the dorsal and anal fins. In *A. charrua*, the dark bands are equal or wider than the lighter spaces between bands, and the design of the unpaired fins consist of light spots over a dark background (Loureiro and García, 2008). These species are a very promising model for investigating the role of sexual selection in reproductive isolation and elucidating mechanisms underlying divergence and speciation in this lineage. We used choice experiments to investigate reproductive isolation between *A. reicherti* and *A. charrua*. In both species, females preferred to mate with their conspecific males, while males did not discriminate between females. Moreover, heterospecific discrimination by females was stronger in areas of sympatry than allopatry

(C. Passos et al., unpublished data). Currently, we are investigating the dimensionality of mate choice underlying reproductive isolation by identifying the cues that mediate assorted mating among species. Hence, determining the traits that underlie assortative mating will allow us to understand how reproductive isolation emerges during speciation and how it is maintained.

Nothobranchius furzeri and *N. orthonotus* are closely related species (Dorn et al., 2014) that regularly occur syntopically. Given the relatively smaller role that female choice plays in the reproductive system of *Nothobranchius* compared to *Austrolebias*, females may be forced to heterospecific matings. It is notable that heterospecific mating may have been driven by male coercion, that is, large *N. orthonotus* males were able to coerce mating from *N. furzeri* females, while spawning in the opposite combination was difficult to induce experimentally (Polačik and Reichard, 2011). The cost to females from a heterospecific mating has been suggested to be lower than the potential cost from male aggression when a female refuses to spawn (Polačik and Reichard, 2011). However, in wild populations females may have more opportunities to escape male attention than under laboratory conditions. Despite that, heterospecific mating was observed in the wild, and in the laboratory the F1, F2, and backcross progeny between *N. furzeri* and *N. orthonotus* is at least partly viable (Polačik and Reichard, 2011). Several *Nothobranchius* individuals of potentially hybrid origin (based on phenotypic traits) were also collected in the wild and await confirmation by genotyping. The sister pair of species, *N. furzeri* and *N. kadleci*, readily hybridize in the laboratory (Ng'oma et al., 2014). The two species have allopatric distributions but would likely naturally hybridize to an extent similar to *A. reicherti* and *A. charrua* should they come into secondary contact. In contradiction to previous assumptions (Dorn et al., 2011), a recent advanced calibration of its phylogenetic tree proposes that most *Nothobranchius* species have a Quaternary origin (Dorn et al., 2014). If this is true, range shifts and speciation in *Nothobranchius* are very dynamic, and prezygotic barriers to heterospecific mating in areas of secondary sympatry are crucial for reproductive isolation (or the lack of it). Annual fishes can therefore become a very useful group for studies on the role of sexually selected reproductive isolation in secondary sympatry, as opposed to the sympatric divergence implicated in radiation of lacustrine fish species (Seehausen et al., 2008).

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